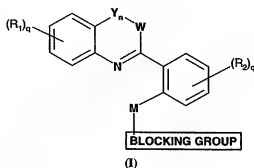


## AMENDMENTS

### In the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Currently Amended) An enzyme substrate of the formula (I):



and biologically acceptable salt, and pro-reporter molecules thereof;

wherein

Y is C=O, and

n is 1 or 0;

W is  $[-N(R_3)-]_n-N(R_3)-$ ;

M is oxygen;

$[[R_1]]R_1$  and  $[[R_2]]R_2$  are, each independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfinio, sulfo, cyano, amino,  $R_4$ -,  $R_4O$ -,  $R_4C(=Z)$ -,  $R_4X-C(=Z)$ -,  $R_4C(=Z)-X$ -,  $R_4X-C(=Z)-Q$ -,  $R_4S$ -,  $R_4S(=O)$ -,  $R_4S(=O)-O$ -,  $R_4S(=O)_2$ -,  $R_4O-S$ -,  $R_4O-S(=O)$ -,  $R_4O-S(=O)_2$ -,  $R_4R_5N-S(=O)$ -,  $R_4R_5N-S(=O)_2$ -,  $R_4R_5N$ -,  $[R_4-C(=Z)][R_5]N$ -,  $[R_4-C(=Z)][R_5-C(=X)]N$ -,  $R_4R_5N-C(=Z)$ -,  $R_4R_5N-C(=Z)-X$ -,  $[R_4R_5N-C(=Z)][R_6]N$ -,  $[R_4R_5N-C(=Z)][R_6-C(=X)]N$ -,  $[R_4-S(=O)][R_5]N$ -,  $[R_4-S(=O)_2][R_5]N$ -,  $(R_4X)(R_5Q)P(=Z)$ -,  $(R_4R_5N)(R_6X)P(=Z)$ -,  $(R_4R_5N)(R_6R_7N)P(=Z)$ -,  $(R_4X)-(R_5Q)P(=Z)-O$ -,  $(R_4R_5N)(R_6X)P(=Z)-O$ -,  $(R_4R_5N)(R_6R_7N)P(=Z)-O$ -,  $(R_4X)-(R_5Q)P(=Z)-S$ -,  $(R_4R_5N)(R_6X)P(=Z)-S$ -,  $(R_4R_5N)(R_6R_7N)P(=Z)-S$ -,  $[(R_4X)(R_5Q)P(=Z)][R_6]N$ -,  $[(R_4R_5N)(R_6X)P(=Z)][R_7]N$ -,  $[(R_4R_5N)(R_6R_7N)P(=Z)][R_8]N$ -,  $(R_4)(R_5X)P(=Z)-O$ -,  $(R_4)(R_5R_6N)P(=Z)-O$ -,  $(R_4)(R_5X)P(=Z)-S$ -,  $(R_4)(R_5R_6N)P(=Z)-S$ -,  $[(R_4)(R_5X)-P(=Z)][R_6]N$ -, or  $[(R_4)(R_5R_6N)P(=Z)][R_7]N$ ;

wherein X, Z and Q are each independently oxygen or sulfur ;

$R_3$  is  $R_4$ ,  $R_4-C(=Z)-$ ,  $R_4X-C(=Z)-$ ,  $R_4R_5N-C(=Z)-$ ,  $R_4O-S(=O)-$ ,  $R_4O-S(=O)_2-$ ,  $R_4R_5N-S(=O)-$ ,  $R_4R_5N-S(=O)_2-$ ,  $(R_4X)(R_5Q)P(=Z)-$ ,  $(R_4R_5N)(R_6X)P(=Z)-$ , or  $(R_4R_5N)(R_6R_7N)P(=Z)-$ ;

wherein  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$  and  $R_8$  are, each independently, hydrogen,  $C_{1-8}$ alkyl,  $C_{2-8}$ alkenyl,  $C_{2-8}$ alkynyl,  $C_{3-7}$ cycloalkyl, aryl,  $Het^1$ , or  $Het^2$ ;

each q is independently 0, 1, 2, 3, or 4;

wherein any  $C_{1-8}$ alkyl,  $C_{2-8}$ alkenyl,  $C_{2-8}$ alkynyl or amino group, may be further mono, di-, or tri-substituted (if the valency allows it) with  $C_{1-4}$ alkoxy,  $C_{1-4}$ alkylcarbonyl,  $C_{1-4}$ alkoxycarbonyl,  $C_{1-4}$ alkylthio,  $C_{1-4}$ alkylsulfonyl,  $C_{1-4}$ alkylsulfenyl,  $C_{1-4}$ alkylsulfinyl,  $C_{1-4}$ alkylsulfonyl,  $C_{1-4}$ alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, aryl,  $Het^1$  or  $Het^2$  substituents;

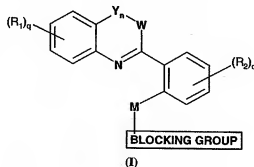
wherein  $Het^1$  is a saturated or partially unsaturated monocyclic, bicyclic or tricyclic heterocycle having preferably 3 to 14 ring members, more preferably 5 to 10 ring members and more preferably 5 to 8 ring members, which contains one or more heteroatom ring members selected from nitrogen, oxygen or sulfur;

wherein  $Het^2$  is an aromatic monocyclic, bicyclic or tricyclic heterocycle having preferably 5 to 14 ring members, more preferably 5 to 10 ring members and more preferably 5 to 6 ring members, which contains one or more heteroatom ring members selected from nitrogen, oxygen or sulfur;

wherein the BLOCKING GROUP is  $\alpha$ -D-glucose,  $\beta$ -D-glucose,  $\alpha$ -D-galactose,  $\beta$ -D-galactose,  $\alpha$ -D-glucose,  $\beta$ -D-glucose,  $\alpha$ -D-galactose,  $\beta$ -D-galactose,  $\alpha$ -D-mannose,  $\beta$ -D-mannose,  $\beta$ -D-N-acetyl-glucosamine,  $\beta$ -D-glucuronic acid,  $\beta$ -D-fucose,  $\alpha$ -L-fucose,  $\alpha$ -L-iduronic acid,  $\beta$ -D-cellobiose,  $\alpha$ -L-arabinose,  $\beta$ -D-xylose,  $\alpha$ -D-N-acetyl-neuraminic acid (sialic acid), aryl esters of p-guanidino benzoic acid, aryl or alkyl phosphate monoesters, aryl sulfate monoesters, aryl phosphates -D-mannose,  $\beta$ -D-mannose,  $\beta$ -D-N-acetyl-glucosamine,  $\beta$ -D-glucuronic acid,  $\beta$ -D-fucose,  $\alpha$ -L-fucose,  $\alpha$ -L-iduronic acid,  $\beta$ -D-cellobiose,  $\alpha$ -L-arabinose,  $\beta$ -D-xylose,  $\alpha$ -D-N-acetyl-neuraminic acid (sialic acid), aryl esters of p-guanidino benzoic acid, aryl or alkyl phosphate monoesters, aryl sulfate monoesters, or aryl phosphates;

with the proviso that at least one  $R_1$ ,  $R_2$  and  $R_3$  is a moiety with at least 4 carbons.

2. (Previously Presented) An enzyme substrate of the formula (I):



and biologically acceptable salts and pro-reporter molecules thereof;

wherein

Y is C=O, and

n is 1 or 0;

W is -N( $R_3$ )-;

M is -O-;

each  $R_1$  and each  $R_2$  present in formula (I) are, independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfinio, sulfo, cyano, amino,  $R_4$ -,  $R_4O$ -,  $R_4-C(=Z)$ -,  $R_4X-C(=Z)$ -,  $R_4-C(=Z)-X$ -,  $R_4X-C(=Z)-Q$ -,  $R_4S$ -,  $R_4-S(=O)$ -,  $R_4-S(=O)_2$ -,  $R_4-S(=O)-O$ -,  $R_4-S(=O)_2-O$ -,  $R_4O-S$ -,  $R_4O-S(=O)$ -,  $R_4O-S(=O)_2$ -,  $R_4R_5N-S(=O)$ -,  $R_4R_5N-S(=O)_2$ -,  $R_4R_5N$ -,  $[R_4-C(=Z)][R_5]N$ -,  $[R_4-C(=Z)][R_5-C(=X)]N$ -,  $[R_4X-C(=Z)][R_5]N$ -,  $[R_4X-C(=Z)][R_5-C(=Q)]N$ -,  $R_4R_5N-C(=Z)$ -,  $R_4R_5N-C(=Z)-X$ -,  $[R_4R_5N-C(=Z)][R_6]N$ -,  $[R_4R_5N-C(=Z)][R_6C(=X)]N$ -,  $[R_4-S(=O)][R_5]N$ -,  $[R_4-S(=O)_2][R_5]N$ -,  $(R_4X)(R_5Q)P(=Z)$ -,  $(R_4R_5N)(R_6X)P(=Z)$ -,  $(R_4R_5N)(R_6R_7N)P(=Z)$ -,  $(R_4X)(R_5Q)P(=Z)-O$ -,  $(R_4R_5N)(R_6X)P(=Z)-O$ -,  $(R_4R_5N)(R_6R_7N)P(=Z)-O$ -,  $(R_4X)(R_5Q)P(=Z)-S$ -,  $(R_4R_5N)(R_6X)P(=Z)-S$ -,  $(R_4R_5N)(R_6R_7N)P(=Z)-S$ -,  $[(R_4X)(R_5Q)P(=Z)][R_6]N$ -,  $[(R_4R_5N)(R_6X)P(=Z)][R_7]N$ -,

$[(R_4R_5N)(R_6R_7N)P(=Z)][R_8]N-$ ,  $(R_4)(R_5X)P(=Z)-O-$ ,  $(R_4)(R_5R_6N)P(=Z)-O-$ ,  $(R_4)(R_5X)P(=Z)-S-$ ,  $(R_4)(R_5R_6N)P(=Z)-S-$ ,  $[(R_4)(R_5X)P(=Z)][R_6]N-$ , or  $[(R_4)(R_5R_6N)P(=Z)][R_7]N-$ ;

wherein X, Z and Q are each, independently, O or S;

$R_3$  is  $R_4$ ,  $R_4-C(=Z)-$ ,  $R_4X-C(=Z)-$ ,  $R_4R_5N-C(=Z)-$ ,  $R_4O-S(=O)-$ ,  $R_4O-S(=O)_2-$ ,  $R_4R_5N-S(=O)-$ ,  $R_4R_5N-S(=O)_2-$ ,  $(R_4X)(R_5Q)P(=Z)-$ ,  $(R_4R_5N)(R_6X)P(=Z)-$ , or  $(R_4R_5N)(R_6R_7N)P(=Z)-$ ;

wherein  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$  and  $R_8$  are each, independently, hydrogen,  $C_{1-8}$ alkyl,  $C_{2-8}$ alkenyl,  $C_{2-8}$ alkynyl,  $C_{3-7}$ cycloalkyl, aryl, Het<sup>1</sup>, or Het<sup>2</sup>;

wherein Het<sup>1</sup> is a saturated or partially unsaturated monocyclic, bicyclic or tricyclic heterocycle having preferably 3 to 14 ring members, more preferably 5 to 10 ring members and more preferably 5 to 8 ring members, which contains one or more heteroatom ring members selected from nitrogen, oxygen or sulfur;

wherein Het<sup>2</sup> is an aromatic monocyclic, bicyclic or tricyclic heterocycle having preferably 5 to 14 ring members, more preferably 5 to 10 ring members and more preferably 5 to 6 ring members, which contains one or more heteroatom ring members selected from nitrogen, oxygen or sulfur;

each q present in formula (I) is, independently, 0, 1, 2, 3, or 4;

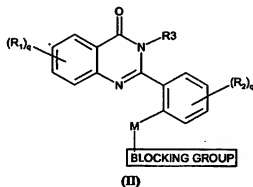
wherein any  $C_{1-8}$ alkyl,  $C_{2-8}$ alkenyl,  $C_{2-8}$ alkynyl or amino group, may be further mono, di-, or tri-substituted (if the valency allows it) with  $C_{1-4}$ alkoxy,  $C_{1-4}$ alkylcarbonyl,

$C_{1-4}$ alkoxycarbonyl,  $C_{1-4}$ alkylthio,  $C_{1-4}$ alkylsulfenyl,  $C_{1-4}$ alkylsulfenyl,  $C_{1-4}$ alkylsulfonyl,  $C_{1-4}$ alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, aryl, Het<sup>1</sup> or Het<sup>2</sup> substituents;

wherein the BLOCKING GROUP is  $\alpha$ -D-glucose,  $\beta$ -D-glucose,  $\alpha$ -D-galactose,  $\beta$ -D-galactose,  $\alpha$ -D-glucose,  $\beta$ -D-glucose,  $\alpha$ -D-galactose,  $\beta$ -D-galactose,  $\alpha$ -D-mannose,  $\beta$ -D-mannose,  $\beta$ -D-N-acetyl-glucosamine,  $\beta$ -D-glucuronic acid,  $\beta$ -D-fucose,  $\alpha$ -L-fucose,  $\alpha$ -L-iduronic acid,  $\beta$ -D-cellobiose,  $\alpha$ -L-arabinose,  $\beta$ -D-xylose,  $\alpha$ -D-N-acetyl-neuraminic acid

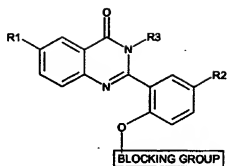
(sialic acid), aryl esters of p-guanidino benzoic acid, aryl or alkyl phosphate monoesters, aryl sulfate monoesters, aryl phosphates -D-mannose,  $\beta$ -D-mannose,  $\beta$ -D-N-acetyl-glucosamine,  $\beta$ -D-glucuronic acid,  $\beta$ -D-fucose,  $\alpha$ -L-fucose,  $\alpha$ -L-iduronic acid,  $\beta$ -D-cellobiose,  $\alpha$ -L-arabinose,  $\beta$ -D-xylose,  $\alpha$ -D-N-acetyl-neuraminic acid (sialic acid), aryl esters of p-guanidino benzoic acid, aryl or alkyl phosphate monoesters, aryl sulfate monoesters, or aryl phosphates; with the proviso that at least one of  $R_1$ ,  $R_2$  and  $R_3$  is  $C_{4-8}$ alkyl,  $C_{4-8}$ alkenyl, or  $C_{4-8}$ alkynyl.

3. (Previously Presented) A substrate according to claim 1, wherein at least one of  $R_1$ ,  $R_2$  and  $R_3$  is independently chosen from the group consisting of straight and branched butyl, pentyl, hexyl, heptyl, or octyl.
4. (Currently Amended) A substrate according to claim 1, wherein W is  $[-N(R_3)-]_n-N(R_3)-$ , Y is  $-C(=O)-$ , and n is 1 and having the formula (II)



wherein M,  $R_1$ ,  $R_2$ ,  $R_3$ , q and the BLOCKING GROUP are as defined as in claim 1.

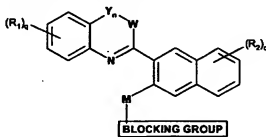
5. (Previously Presented) A substrate according to claim 1, having the formula (III)



(III)

wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and the BLOCKING GROUP are as defined in claim 1.

6. (Withdrawn) An enzyme substrate of the formula (V):



(V)

and biologically acceptable salts, and pro-reporter molecules thereof ;

wherein

Y is  $\text{>C=O}$ ,  $\text{>C=CH}_2$ ,  $\text{>C=R1}$ , and

n is 1 or 0;

W is  $\text{=CH-}$ ,  $\text{-S-}$ ,  $\text{-O-}$ , or  $\text{-N(R3)-}$ ;

M is  $\text{-O-}$ ,  $\text{-N(R3)-}$ , or  $\text{-S-}$ ;

each R<sub>1</sub> and each R<sub>2</sub> present in formula (V) are, independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfinio, sulfo, cyano, amino, R<sub>4</sub>-, R<sub>4</sub>O-, R<sub>4</sub>C (=Z)-, R<sub>4</sub>X-C (=Z)-, R<sub>4</sub>C (=Z) -X-, R<sub>4</sub>X-C (=Z)-Q-, R<sub>4</sub>S-, R<sub>4</sub>S (=O)-, R<sub>4</sub>S (=O) 2-, R<sub>4</sub>S (=O)-O-, R<sub>4</sub>S (=O) 2-O-, R<sub>4</sub>O-S-, R<sub>4</sub>O-S(=O)-, R<sub>4</sub>O-S(=O)2-, R<sub>4</sub>R<sub>5</sub>N-S(=O)-, R<sub>4</sub>R<sub>5</sub>N-S(=O)2-, R<sub>4</sub>R<sub>5</sub>N-, [R<sub>4</sub>-C(=Z)][R<sub>5</sub>N-], [R<sub>4</sub>-C(=Z)][R<sub>5</sub>-C(=X)]N-, [R<sub>4</sub>X-C(=Z)][R<sub>5</sub>N-], [R<sub>4</sub>X-C(=Z)][R<sub>5</sub>-C(=Q)]N-, R<sub>4</sub>R<sub>5</sub>N-C (=Z) -, R<sub>4</sub>R<sub>5</sub>N-C (=Z)-X- f<sub>8</sub>N-C (=Z) ] gt6] N-, [R<sub>4</sub>R<sub>5</sub>N-C (=Z)] [R<sub>6</sub>c (=X)]N-, [R<sub>4</sub>-

$S(=O)][R_5]N-$ ,  $[R_4-S(=O)_2][R_5]N-$ ,  $(R_4X)(R_5Q)P(=Z)-$ ,  $(R_4R_5N)(R_6X)P(=Z)-$ ,  
 $(R_4R_5N)(R_6R_7N)P(=Z)-$ ,  $(R_4X)(R_5Q)P(=Z)-O-$ ,  $(R_4R_5N)(R_6X)P(=Z)-O-$ ,  
 $(R_4R_5N)(R_6R_7N)P(=Z)-O-$ ,  $(R_4X)(R_5Q)P(=Z)-S-$ ,  $(R_4R_5N)(R_6X)P(=Z)-S-$ ,  
 $(R_4R_5N)(R_6R_7N)P(=Z)-S-$ ,  $<BR> [(R_4X)(R_5Q)P(=Z)][R_6]N-$ ,  $[(R_4R_5N)(R_6X)P(=Z)][R_7]N-$ ,  
 $[(R_4R_5N)(R_6R_7N)P(=Z)][R_8]N-$ ,  $(R_4)(R_5X)P(=Z)-O-$ ,  $(R_4)(R_5R_6N)P(=Z)-O-$ ,  
 $(R_4)(R_5X)P(=Z)-S-$ ,  $(R_4)(R_5R_6N)P(=Z)-S-$ ,  $[(R_4)(R_5X)P(=Z)][R_6]N-$ ,  
 $[(R_4)(R_5R_6N)P(=Z)][R_7]N-$ ;

wherein the  $R_2$  substituent can replace one or more hydrogens on any carbon atom of the naphthyl group, such as carbon atoms C1, C4, C5, C6, C7, and C8, provided that the carbon's valency is not exceeded; wherein X, Z and Q are each, independently, O or S;

$R_3$  is  $R_4$ ,  $R_4-C(=Z)-$ ,  $R_4X-C(=Z)-$ ,  $R_4R_5N-C(=Z)-$ ,  $R_4O-S(=O)-$ ,  $R_4O-S(=O)-2-$ ,  $R_4R_5N-S(=O)-$ ,  $R_4R_5N-S(=O)_2-$ ,  $(R_4X)(R_5Q)P(=Z)-$ ,  $(R_4R_5N)(R_6X)P(=Z)-$ ,  $(R_4R_5N)(R_sR_n)P(=Z)-$ ;

wherein  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$  and  $R_a$  are each, independently, hydrogen, C1-8alkyl, C2-8alkenyl, C2-8alkynyl, C3-7cycloalkyl, aryl, Het1, Het2 ;

each q present in formula (V) is, independently, 0, 1, 2, 3, or 4;

wherein any C1-8alkyl, C2-8alkenyl, C2-8alkynyl or amino group, may be further mono, di-, or tri-substituted (if the valency allows it) with C1-4alkoxy, C1-4alkylcarbonyl, C1-4alkoxycarbonyl, C1-4alkylthio, C1-4alkylsulfenyl, C1-4alkylsulfinyl, C1-4alkylsulfonyl, C1-4alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, aryl, Het1 or Het2 substituents;

wherein the BLOCKING GROUP is a mono- or polysaccharide derivate, phosphate derivate, sulfate derivate, carboxylic acid derivate, or oligopeptide derivate;

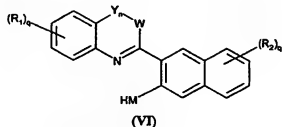
with the proviso that at least one of  $R_1$ ,  $R_2$  and  $R_3$  is C4-8alkyl, C4-8alkenyl, or C4-8alkynyl.

7. (Withdrawn) Use of a substrate according to claim 1, for permeation through the membrane of a biological cell.

8. (Withdrawn) Method for preparing a substrate according to claim 1, comprising the steps of:

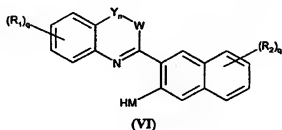
- synthesizing a blocking group, whereby said blocking group may be optionally protected;
- synthesizing a substituted fluorophore;
- coupling the optionally protected blocking group to said substituted fluorophore;
- optionally deprotecting said blocking group; and
- purifying the resulting substituted substrate.

9. (Withdrawn) A fluorescent precipitate obtainable by cleavage of the BLOCKING GROUP moiety from the substrate of formula (I) of any claim 1, having the formula (IV)



wherein Y, n, W, M, R<sub>1</sub>, R<sub>2</sub> and q are as defined as in claim 1.

10. (Withdrawn) A fluorescent precipitate obtainable by cleavage of the BLOCKING GROUP moiety from the substrate of formula (V) of claim 6, having the formula (VI)



wherein Y, n, W, M, R<sub>1</sub>, R<sub>2</sub> and q are as defined as in claim 6.

11. (Withdrawn) Method for detecting the activity of an enzyme comprising the steps of: -
- contacting a sample containing said enzyme with a substrate according to claim 1;
  - applying conditions suitable to allow formation of a fluorescent precipitate;
- said fluorescent precipitate comprising a fluorescent precipitate obtained by cleavage of the BLOCKING GROUP moiety from the substrate of formula (I) of claim 1, having formula (IV)

wherein Y, n, W, M, R1, R2 and q are defined as claim 1; and

- quantitatively or qualitatively analyzing said fluorescent precipitate.

-

12. (Withdrawn) Method according to claim 11 wherein analyzing said fluorescent precipitate comprises the steps of:

- exposing the fluorescent precipitate to a light source capable of producing light at a wavelength of absorption of the fluorescent precipitate; and
- detecting the resultant fluorescence of the precipitate.